Mycorrhizal mediation of plant N acquisition and residue decomposition: Impact of mineral N inputs

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Abstract

Mycorrhizas are ubiquitous plant–fungus mutualists in terrestrial ecosystems and play important roles in plant resource capture and nutrient cycling. Sporadic evidence suggests that anthropogenic nitrogen (N) input may impact the development and the functioning of arbuscular mycorrhizal (AM) fungi, potentially altering host plant growth and soil carbon (C) dynamics. In this study, we examined how mineral N inputs affected mycorrhizal mediation of plant N acquisition and residue decomposition in a microcosm system. Each microcosm unit was separated into HOST and TEST compartments by a replaceable mesh screen that either prevented or allowed AM fungal hyphae but not plant roots to grow into the TEST compartments. Wild oat (_Avena fatua_ L.) was planted in the HOST compartments that had been inoculated with either a single species of AM fungus, _Glomus etunicatum_, or a mixture of AM fungi including _G. etunicatum_. Mycorrhizal contributions to plant N acquisition and residue decomposition were directly assessed by introducing a mineral ^{15}N tracer and ^{13}C-rich residues of a C_4 plant to the TEST compartments. Results from ^{15}N tracer measurements showed that AM fungal hyphae directly transported N from the TEST soil to the host plant. Compared with the control with no penetration of AM fungal hyphae, AM hyphal penetration led to a 125% increase in biomass ^{15}N of host plants and a 20% reduction in extractable inorganic N in the TEST soil. Mineral N inputs to the HOST compartments (equivalent to $5.0 \, \mathrm{g \, N \, m^{-2} \, yr^{-1}}$) increased oat biomass and total root length colonized by mycorrhizal fungi by 189% and 285%, respectively, as compared with the no-N control. Mineral N inputs to the HOST plants also reduced extractable inorganic N and particulate residue C proportion by 58% and 12%, respectively, in the corresponding TEST soils as compared to the no-N control, by stimulating AM fungal growth and activities. The species mixture of mycorrhizal fungi was more effective in facilitating N transport and residue decomposition than the single AM species. These findings indicate that low-level mineral N inputs may significantly enhance nutrient cycling and plant resource capture in terrestrial ecosystems via stimulation of root growth, mycorrhizal functioning, and residue decomposition. The long-term effects of these observed alterations on soil C dynamics remain to be investigated.

Keywords: arbuscular mycorrhizal fungi, ^{13}C tracer, decomposition, hyphal N transport, mineral N inputs, ^{15}N tracer, particulate organic C, plant N acquisition, soil C

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Introduction

Arbuscular mycorrhizal (AM) fungi form mutualistic symbioses with the roots of plants. More than 80% of

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the higher plant species examined have been reported to be associated with AM fungi (Smith & Read, 1997). These symbiotic associations are particularly important in plant acquisition of less mobile mineral nutrients such as phosphorus (P) and zinc (Zn), and to some extent other ions such as potassium (K), calcium (Ca), and magnesium (Mg) (Clark & Zeto, 2000). Although AM fungi have been shown to take up and transport N from soil to plants (Ames *et al.*, 1983; Bago *et al.*, 1996; Mäder *et al.*, 2000), their importance in plant N nutrition has not been fully appreciated until recently (Read & Perez-Moreno, 2003). This can at least partially be attributed to the assumption that AM fungi have little capacity to enhance plant uptake of mobile ions such as NO_3^- because these ions move rapidly in soil (Tinker & Nye, 2000).

In addition to facilitating plant uptake of mineral nutrients, AM fungi can impact soil C dynamics directly by contributing to soil C inputs (Jakobsen & Rosendahl, 1990), and indirectly by modifying the decomposition of soil organic materials. AM fungal hyphae and secondary compounds (particularly exopolysaccharides and glomalin) can facilitate the formation and stabilization of soil aggregates (Miller & Jastrow, 1990; Tisdall *et al.*, 1997; Wright & Upadhyaya, 1998), thus reducing microbial degradation of organic matter (OM). Although AM fungi are thought to be unable to decompose OM directly due to a lack of saprotrophic capacity (Read & Perez-Moreno, 2003), they may still be involved in decomposition processes. AM fungi have been found to proliferate in decomposing organic residues (St John *et al.*, 1983), and therefore, likely enhance residue decomposition by stimulating the activity of saprophytic microbes (Hodge *et al.*, 2001).

Human activities have significantly increased N inputs to terrestrial ecosystems through mineral N fertilization and atmospheric N deposition, modifying the global N cycle (Chapin *et al.*, 2002). Nitrogen inputs to some crop fields through chemical fertilizers have been as high as $30 \, g \, N \, m^{-2} \, yr^{-1}$ (USDA-NASS, 2004), leading to a total input of about $80 \, Tg \, N \, yr^{-1}$ to global agroecosystems (Fixen & West, 2002). Atmospheric reactive N deposition is estimated to range from 0.5 to $2.5 \, g \, N \, m^{-2} \, yr^{-1}$ in eastern North America and from 0.5 to $6.0 \, g \, N \, m^{-2} \, yr^{-1}$ in northern Europe (Wedin & Tilman, 1996). Elevated N deposition has been associated with increases in plant primary productivity, alterations in plant species composition, and reductions in plant diversity (Wedin & Tilman, 1996; Gough *et al.*, 2000). Nitrogen-induced changes in plants may profoundly impact AM symbionts because AM fungi depend on host plants for C supply (Smith & Read, 1997; Nakano *et al.*, 2001). Some studies have shown that increased N inputs negatively impact AM fungi by

suppressing their abundance and functioning (Buwalda & Goh, 1982; Egerton-Warburton & Allen, 2000; Corkidi *et al.*, 2002). A meta-analysis of 17 AM studies by Treseder (2004) has recently shown that N additions reduced mycorrhizal fungal abundance by an average of 15%. However, other experiments have documented enhancement of AM fungi by N inputs. For example, Dhillion & Ampornpan (1992) found that N addition stimulated root colonization of rice plants by vesicular-arbuscular mycorrhizal fungi. Application of NH_4NO_3 also increased root colonization of *Leucaena leucocephala* by *Glomus aggregatum* (Aziz & Habte, 1989). Treseder & Allen (2002) observed direct N limitation of AM fungi in three rain forests in Hawaii and found enhancement of AM fungal biomass by N applications in low N soils. These discrepancies among studies may stem from differences in initial soil nutrient status, climate, and host plants. However, it is unclear whether and how changes in arbuscular mycorrhizal caused by increased N inputs impact mycorrhizal mediation of plant N acquisition and residue decomposition. This information is important for understanding how anthropogenic N inputs affect mineral nutrient acquisition and cycling in terrestrial ecosystems.

We conducted two experiments to examine the effects of mineral N inputs on mycorrhizae, N uptake by host plants, and residue decomposition. The first experiment was designed to examine mycorrhizally mediated N transport and uptake by host plants using a ^{15}N tracer, and the second one aimed to quantify the effects of low-level N inputs on both mycorrhizal root colonization and mycorrhizal mediation of residue decomposition. In addition, we examined whether AM fungal species composition affected mycorrhizal mediation of hyphal N transport and organic decomposition.

Materials and methods

Experimental conditions

The experiments were conducted in the Phytotron facility at North Carolina State University (NCSU), Raleigh, NC, USA. Air temperature in the treatment chamber was maintained at $26 \pm 1 \, °C$ during the day and $22 \pm 1 \, °C$ at night. At plant level, the photosynthetically active radiation flux was $505 \, \mu mol \, m^{-2} \, s^{-1}$ with a day length of 9 h.

We constructed microcosms using plexi-glass (modified from Hodge *et al.* (2001)) to manipulate mycorrhizae, and five of these microcosm units were used as five replicates for each experiment. Each microcosm was divided into six compartments with each compartment measuring $13 \times 14 \times 15 \, cm$ (width $\times$ depth $\times$ height) (Fig. 1). Three compartments in a row were
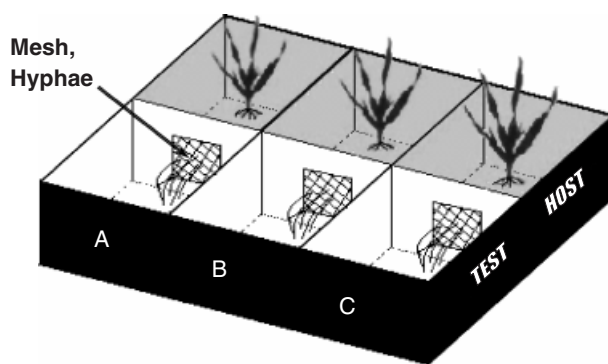
Fig. 1 A microcosm unit composed of six equal-size compartments. Three compartments in one row were assigned as the HOST compartments, and the others as the TEST compartments. When a $0.45\,\mu m$ mesh screen was placed between the HOST and TEST compartments, could neither arbuscular mycorrhizal (AM) hyphae nor plant roots pass through the mesh from the HOST to the TEST compartments. When a $20\,\mu m$ mesh screen was placed between the two compartments, only AM hyphae were allowed to pass through the mesh from the HOST to the TEST compartments.

designated as HOST compartments (containing host plants and AM fungi) and the three adjacent compartments were designated TEST compartments to assess mycorrhizal functioning. The HOST and TEST compartments were separated by a replaceable 20 or $0.45\,\mu m$ mesh fabric panel (Tetko/Sefar mesh, Sefar America, NY) that prevented plant roots or both roots and AM fungal hyphae from growing into the TEST compartments, respectively (Hodge *et al.*, 2001). Effectiveness of the $20\,\mu m$ mesh fabric in preventing root growth into the TEST compartment was visually assessed at the completion of each experiment. Each compartment of the microcosm unit was filled with $3.5\,kg$ of an autoclaved quartz sand and sandy loam soil ($1:1$ w/w) mixture. The soil used in this study was collected from a long-term C_3 grassland and had a $\delta^{13}C$ value of $-25.66‰$. The total C and N concentrations in the growth medium were 9.20 and $0.4\,g\,kg^{-1}$, respectively, and extractable inorganic N was $17.6\,mg\,kg^{-1}$.

Experiment 1: Nitrogen uptake and transport by AM fungal hyphae

This experiment had three treatments: (1) a single AM fungal species without fungal hyphal access to the TEST soil (AMF−H) (control), (2) a single AM fungal species with hyphal access to the TEST soil (AMF + H), and (3) a multiple AM fungal species with hyphal access to the TEST soil (AMFs + H). In the AMF−H control, the HOST and TEST compartments were separated by a fine mesh screen ($0.45\,\mu m$) that prevented access by

both plant roots and AM fungal hyphae to the TEST compartment soil. In the AMF + H and AMFs + H treatments, a $20\,\mu m$ mesh screen was used to allow AM fungal hyphae, but not plant roots to grow into the TEST compartment.

The single AM fungal species, *Glomus* aff. *etunicatum* Becker & Gerdemann, was originally trap-cultured from an agricultural soil collected from the Central Crops Research Station, NC. The fungus was subsequently pot-cultured from a single spore, as a single AM fungal species. The multiple AM fungal species were trap-cultured from an agricultural soil, collected from the Center for Environmental Farming Systems, NC, and was then pot-cultured to increase fungal biomass. Twelve AM fungal species were identified and characterized according to the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM) at http://invam.caf.wvu.edu (Table 1). AM fungal inoculum consisted of culture media containing spores, hyphae, and colonized root pieces.

At the beginning of the experiment, $3.0\,mg\,kg^{-1}$ soil of ^{15}N (($NH_4)_2SO_4$, 99.4 at % ^{15}N) was applied to all TEST compartments. Nitrogen transport by AM fungal hyphae was assessed by determining [^{15}N] in the plants after growth in the HOST compartments for 15 weeks. Wild oat (*Avena fatua* L.) was planted in the HOST compartments in each treatment, and allowed to grow for 15 weeks. Then, the plants were harvested, dried, weighed, and analyzed for N concentrations. Soil subsamples were also collected for analyses.

A bioassay method was used to assess the presence of AM fungal hyphae and N status in the TEST compartments. Four corn (*Zea mays* L.) caryopses were planted in each TEST compartment 12 weeks after the experiment began. Three weeks later, plants in both HOST and TEST compartments were collected for biomass determinations and N analyses. Soil samples from both compartments were also collected for analyses.

Experiment 2: The effects of mineral N inputs on AM fungal colonization and AM-mediated residue decomposition

In this experiment, the host plant, AM fungal inoculations, and planting medium were identical to those in Experiment 1. However, all the HOST and TEST compartments were separated by a $20\,\mu m$ mesh screen that allowed AM fungal hyphae but not plant roots to penetrate the TEST compartments. The three treatments applied to the HOST compartments in each microcosm unit were: (1) single AM fungal species without N addition (AMF−N) (control), (2) single AM fungal species with N addition (AMF + N), and (3) multiple

Table 1 Putative AM fungal species composition of the AM fungal species mixture used in experiment

Genera	Species
Acaulospora	*Acaulospora* aff. *koskei* Blaszkowski
	Acaulospora aff. *mellea* Spain & Schenck
	Acaulospora aff. *scrobiculata* Trappe
	Acaulospora sp. gold-brown with a reticulate outer wall
Entrophospora	*Entrophospora* aff. *infrequens* (Hall) Ames & Schneider
Gigaspora	*Gigaspora* aff. *margarita* Becker & Hall
Glomus	*Glomus* aff. *clarum* Nicolson & Schenck
	Glomus aff. *etunicatum* Becker & Gerdemann
	Glomus aff. *fasciculatum* (Thaxter) Gerdemann & Trappe emend. Walker
	Glomus aff. *intraradices* Schenck & Smith
Paraglomus	*Paraglomus occultum* (?) (Walker) Morton & Redecker
Scutellospora	*Scutellospora* aff. *calospora* (Nicolson & Gerdemann) Walker & Sanders

AM fungal species mixture with N addition (AMFs + N).

To minimize the impact of inorganic N on the initial colonization of mycorrhizal fungi, mineral N of $12.5\,mg\,N\,kg^{-1}$ soil (equivalent to $2.5\,g\,N\,m^{-2}$) as NH_4NO_3 was applied to the HOST plants in the AMF + N and AMFs + N treatments at the 6th and 8th week after seedling emergence. The plants were allowed to grow for 15 weeks, and then harvested, dried, weighed, and analyzed for N concentration. Also, a soil sample from each compartment was collected for laboratory analyses.

To assess the effects of N inputs on residue decomposition, 35.0 g of dried and chopped (<1 cm length) shoot tissue of switchgrass (*Panicum virgatum* L.), a C_4 plant that is enriched in ^{13}C as compared with C_3 plants, was thoroughly mixed with the sand/soil medium in the TEST compartments of each treatment replicate at the beginning of the experiment. One subsample (referred as the initial soil sample) was collected from each treatment replicate immediately after amendment with the ^{13}C labeled residue. The $\delta^{13}C$ value of the added residues was −12.2‰. The loss of the residue C through mineralization in the TEST compartments was estimated by measuring the difference in soil $\delta^{13}C$ values between the beginning and end of the 15 week experiment.

Mycorrhizal root colonization

Colonization of roots by mycorrhizal fungi was measured on roots stained with trypan blue (Phillips & Hayman, 1970), using the gridline-intersect method (Giovannetti & Mosse, 1980). Thoroughly washed root samples (cut into about 1 cm in length) were cleared in 5% (w/v) KOH, acidified in 1% (v/v) HCl, and then stained with acidic glycerol-trypan blue solution. The stained roots were then spread on a Petri dish with gridlines and examined for infection using a dissecting microscope at ×40 magnification. Results obtained were expressed as percentage root length colonized (PRLC) and total root length colonized (TRLC) by AM fungi.

Nitrogen determinations

Total N concentrations in finely milled plant tissues were determined using a Perkin-Elmer 2400 CHNS/O elemental analyzer (Norwalk, CT, USA). ^{15}N in milled plant tissues was measured using a Thermo Finnigan DELTAPlus continuous flow isotope ratio mass spectrometer (CF-IRMS, Bremen, Germany). Soil inorganic N was estimated as described previously (Hart *et al.*, 1994), using 1.0 M KCl as the extraction solution and a fluid injection auto-analyzer (Lachat Instruments, Milwaukee, WI, USA) to measure N concentration.

Fractionation and determination of OM

Soil samples collected before residue incorporation, immediately following the residue incorporation (the initial soil), and after incubation were subject to OM fractionation. Density fractionation of soil OM was performed using a procedure modified from Baisden & Amundson (2002). Briefly, 10.0 g soil samples were first extracted with 50 mL of distilled water in 100 mL flasks. After gentle dispersal by hand, the flasks were left standing overnight at room temperature. The first fraction (F1, light OM with $d < 1.0\,g\,cm^{-3}$) was collected by filtration of the supernatant through Whatman No.1 filter paper. The sediment in the flasks was resuspended in 50 mL of sodium polytungstate solution ($d = 1.6\,g\,cm^{-3}$) by hand-stirring. The suspension was allowed to stand at room temperature for at least 1 hr.

The supernatant containing OM with $d < 1.6\,\text{g cm}^{-3}$ was collected by filtration as the second fraction (F2, heavy OM), and the residue was regarded as the third fraction (F3, very heavy OM with $d > 1.6\,\text{g cm}^{-3}$). All fractions were oven-dried at $65\,^{\circ}\text{C}$ and ground to a fine powder before ^{13}C determination on a Thermo Finnigan DELTAPlus continuous flow isotope ratio mass spectrometer (CF-IRMS, Bremen, Germany).

The mean proportion (f_R) of the residue C in the whole soil or soil fractions and the standard error (SE) of the f_R can be calculated using the single isotope, two-source mixing model (Phillips & Gregg, 2001) as

$$f_R = (\delta_T - \delta_S)/(\delta_R - \delta_S),$$

$$SE_{f_R} = \sqrt{[\sigma^2_{\delta_T} + f^2_R \sigma^2_{\delta_R} + (1 - f_R)^2 \sigma^2_{\delta_S}]/(\delta_R - \delta_S)^2},$$

where δ_R and $\sigma^2_{\delta_R}$ represent the mean $\delta^{13}\text{C}$ value and its variance for the residue, respectively. δ_S and $\sigma^2_{\delta_S}$ refer to the mean $\delta^{13}\text{C}$ value and its variance for the initial soil in the whole soil or the soil fractions, respectively. δ_T and $\sigma^2_{\delta_T}$ are the mean $\delta^{13}\text{C}$ value and its variance for the treated soil in the whole soil or the soil fractions, respectively.

Statistical analyses

The experiment was a randomized complete block with five replicates per treatment. Microcosm units were treated as blocks in the analysis. Treatment effects were statistically analyzed using analysis of variance techniques (PROC MIXED, SAS (Littell *et al.*, 1996)). A least significant difference (LSD) test was conducted on all data. For all tests, differences were considered significant when $P \leq 0.05$.

Results

Experiment 1: Nitrogen uptake and transport by AM fungal hyphae

Mycorrhizally mediated N transport to the plant. In this experiment, we tested mycorrhizally mediated N uptake and transport from the soil to the host plant (wild oat) using a ^{15}N tracer. AM fungal hyphal access to the soil in the TEST compartments significantly enhanced N concentrations in the shoot of wild oat plants compared with no hyphal access to the TEST soil, leading to a 55% and 125% increase in biomass N and ^{15}N, respectively, in the plants in the AMF + H treatment (Table 2). The AM fungal species mixture treatment (AMFs + H) resulted in even higher biomass N and ^{15}N accumulations in the host plants than the single AM fungal species treatment (AMF + H). Total ^{15}N content in the host plants from the AMF−H treatment was very low, indicating little diffusion and mass flow of ^{15}N through the nylon mesh from the TEST to the HOST compartment soil.

Soil extractable inorganic N. Extractable inorganic N in the HOST soils was not significantly different among treatments $(0.6–0.9\,\text{mg N kg}^{-1})$ (Fig. 2). However, extractable inorganic N in the TEST soils was 20% and 70% lower in the AMF + H and AMFs + H, respectively, than in the AMF−H (Fig. 2).

Host plant biomass. Dry mass of wild oat plants was not significantly different between the AMF + H treatment and the AMF−H control (Table 2). But, plant dry mass was 122% higher in the multiple AM fungal species treatment (AMFs + H) than in the AMF−H (Table 2).

Table 2 Biomass, AM fungal infection (PRLC and TRLC), and N contents in the HOST plants (wild oat) as influenced by AM fungal access to the TEST soil at 15 weeks after seedling emergence (Experiment 1)

Treatment	Biomass (g m^{-2})	PRLC (%)	TRLC (m CPT^{-1})	Shoot ^{15}N concentration (Atom %)	Biomass N (mg CPT^{-1})	Biomass ^{15}N (mg CPT^{-1})
AMF−H	214 ± 13b*	15 ± 1b	46 ± 7b	0.49 ± 0.03c	25.6 ± 1.7c	0.12 ± 0.01c
AMF + H	271 ± 28b	19 ± 2b	61 ± 12b	0.80 ± 0.08b	39.8 ± 4.0b	0.27 ± 0.02b
AMFs + H	497 ± 30a	26 ± 1a	136 ± 19a	1.68 ± 0.09a	67.0 ± 5.2a	0.99 ± 0.10a

*Values are means ± SE; the values followed by the different letters in a column are significantly different at $P \leq 0.05$ (LSD). AMF−H, wild oat and single arbuscular mycorrhizal fungal species in the HOST compartment without oat root or mycorrhizal hyphal access to the TEST compartment; AMF + H, oat and single arbuscular mycorrhizal fungal species in the HOST compartment with only hyphal access to the TEST compartment; AMFs + H, oat and multiple arbuscular mycorrhizal fungal species in the HOST compartment with only hyphal access to the TEST compartment; PRLC and TRLC, percentage of, and total root length colonized by arbuscular mycorrhizal fungi, respectively; CPT, compartment; AM, arbuscular mycorrhizal; LSD, least significant difference.

Mycorrhizal root colonization. The total root length of wild oat colonized (TRLC) and percent root length colonized (PRLC) by AM fungi did not differ significantly between the AMF−H control and AMF + H treatments. However, TRLC and PRLC were respectively 196% and 73% higher in the AMFs + H treatment than in the AMF−H control (Table 2).

Corn bioassay. The corn plants, sown in the TEST soils 12 weeks after the beginning of the experiment, responded differently to the treatments applied to the HOST plants. Plant dry biomass was highest in the AMF−H control, followed by the AMF + H and the AMFs + H

Fig. 2 Extractable inorganic N in the HOST and TEST soils as influenced by the access of arbuscular mycorrhizal hyphae to the TEST soil at 12 weeks after seedling emergence. AMF−H: wild oat and single arbuscular mycorrhizal (AM) fungal species in the HOST compartment without AM fungal hyphal access to the TEST compartment, AMF + H: oat and single AM fungal species with hyphal access to the TEST compartment, and AMFs + H: oat and multiple AM fungal species with hyphal access to the TEST compartment. Bars with different letters are significantly different at $P \leq 0.05$ (least significant difference).

treatments (Table 3). Corn leaves exhibited mild chlorosis in the AMFs + H (data not shown). The PRLC was 58%, 36% and 7% in the AMFs + H, AMF + H, and AMF−H, respectively. Similar to the corn plant biomass results, shoot ^{15}N concentration, biomass N and biomass ^{15}N in corn plants were found to be highest in the AMF−H and lowest in the AMFs + H (Table 3). During excavation of corn plants, the system was examined for evidence that oat roots penetrated the fabric panel, and none was found.

Experiment 2: The effects of mineral N inputs on AM fungal colonization and AM-mediated residue decomposition

Plant growth and mycorrhizal infection. Addition of mineral N to the HOST soils stimulated the growth of wild oat plants. Total dry biomass of oat shoots and roots increased from $210 \pm 4\,\mathrm{g\,m^{-2}}$ in the control (a single AM fungus without N inputs, AMF−N) to $605 \pm 34\,\mathrm{g\,m^{-2}}$ in the treatment with a single AM fungus with N inputs (AMF + N) and $677 \pm 23\,\mathrm{g\,m^{-2}}$ in the multiple AM fungi with N inputs (AMFs + N) treatment (Fig. 3). However, plants in the N enriched soils, i.e. AMF + N and AMFs + N, allocated a greater proportion (50–80%) of their C to shoots compared with the AMF−N control. Again, at the time of excavation of plants, there was no indication that oat roots penetrated the fabric panel between HOST and TEST compartments.

Although N additions did not significantly increase the percentage root length colonized by AM fungi (22–27%), total root length colonized was significantly enhanced in the compartments receiving N compared with the control (200–214 m in the + N treatments vs. 52 m in the control) (Fig. 4). No significant differences
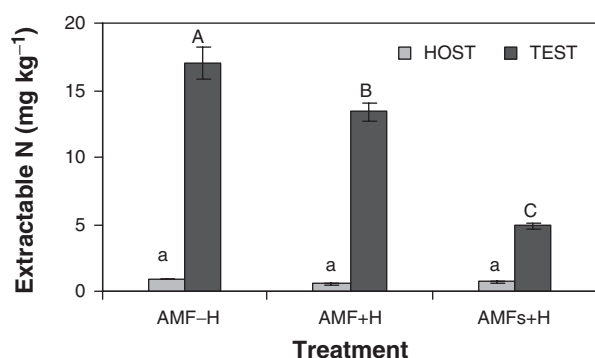
Table 3 Biomass, AM fungal infection (PRLC), and N contents in corn plants grown in the TEST soils during the final three weeks of the experiment (Experiment 1)

Treatment	Biomass (g m^{-2})	PRLC (%)	Shoot ^{15}N concentration (Atom %)	Biomass N (mg CPT^{-1})	Biomass ^{15}N (mg CPT^{-1})
AMF−H	148 ± 8a*	7 ± 2c	1.42 ± 0.02a	39.1 ± 2.5a	0.52 ± 0.07a
AMF + H	124 ± 19a	36 ± 5b	1.09 ± 0.08b	29.7 ± 4.1b	0.33 ± 0.05b
AMFs + H	56 ± 5b	58 ± 2a	0.41 ± 0.01c	12.1 ± 1.3c	0.05 ± 0.01c

*Values are means ± SE; the values followed by different letters in a column are significantly different at $P \leq 0.05$ (LSD).
AMF−H, wild oat and single arbuscular mycorrhizal fungal species in the HOST compartment without oat root or mycorrhizal hyphal access to the TEST compartment; AMF + H, oat and single arbuscular mycorrhizal fungal species in the HOST compartment with only hyphal access to the TEST compartment; AMFs + H, oat and multiple arbuscular mycorrhizal fungal species in the HOST compartment with only hyphal access to the TEST compartment; PRLC, percentage of root length colonized by arbuscular mycorrhizal fungi; CPT, compartment; AM, arbuscular mycorrhizal; LSD, least significant difference.

in PRLC or TRLC were found between the AMF + N and AMFs + N treatments.

Soil extractable inorganic N. At the end of the experiment, extractable inorganic N in the HOST soils was very low ($0.7–0.9 \, \text{mg N kg}^{-1}$) with no statistically significant differences among the treatments. By contrast, extractable inorganic N in the TEST soils varied
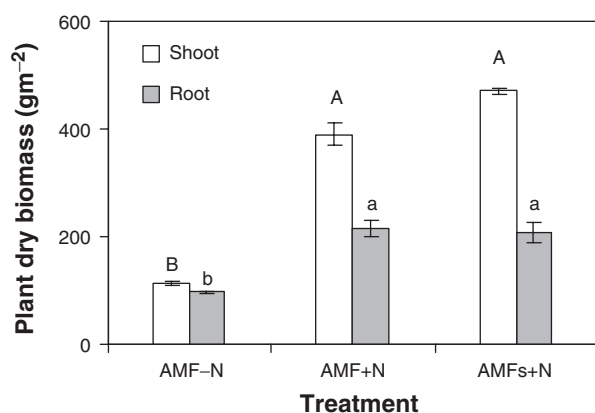

Fig. 3 Effect of mineral N additions as NH_4NO_3 to the HOST soils on shoot and root biomass of wild oats 15 weeks after seedling emergence. AMF−N: wild oat and single arbuscular mycorrhizal (AM) fungal species in the HOST compartment without N inputs, AMF + N: oat and single AM fungal species plus N in the HOST compartment, and AMFs + N: oat and multiple AM fungal species plus N in the HOST compartment. Bars with different letters are significantly different at $P \leq 0.05$ (least significant difference).
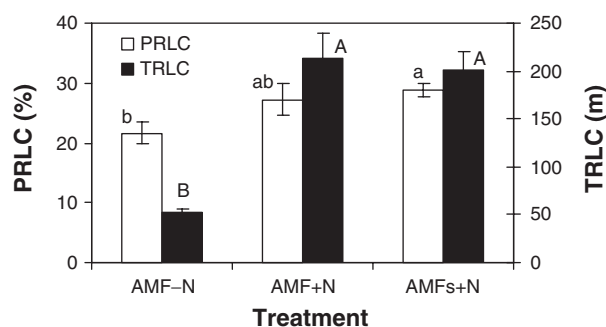

Fig. 4 Effect of mineral N additions as NH_4NO_3 to the HOST soils on percentage root length colonized (PRLC) and total root length colonized (TRLC) by arbuscular mycorrhizae of wild oats plants 15 weeks after seedling emergence. AMF−N: wild oat and single arbuscular mycorrhizal (AM) fungal species in the HOST compartment without N inputs, AMF + N: oat and single AM fungal species plus N in the HOST compartment, and AMFs + N: oat and multiple AM fungal species plus N in the HOST compartment. Bars with different letters are significantly different at $P \leq 0.05$ (least significant difference).
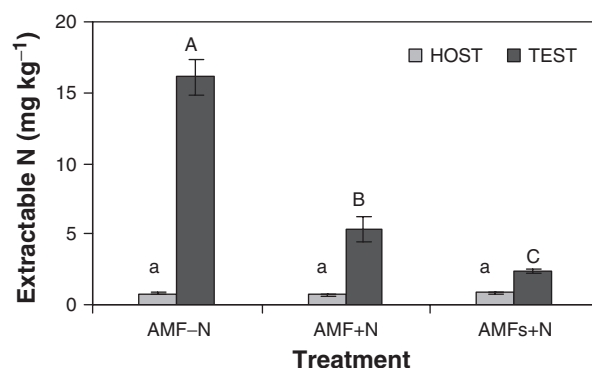

Fig. 5 Effect of mineral N additions as NH_4NO_3 to the HOST soils on extractable inorganic N in the HOST and TEST soils 15 weeks after seedling emergence. AMF−N: wild oat and single arbuscular mycorrhizal (AM) fungal species in the HOST compartment without N inputs, AMF + N: oat and single AM fungal species plus N in the HOST compartment, and AMFs + N: oat and multiple AM fungal species plus N in the HOST compartment. Bars with different letters are significantly different at $P \leq 0.05$ (least significant difference).

significantly among the treatments (Fig. 5). Specifically, extractable N was significantly lower in the AMF + N ($5.4 \pm 0.90 \, \text{mg N kg}^{-1}$) and AMFs + N ($2.4 \pm 0.19 \, \text{mg N kg}^{-1}$) than in the AMF−N control ($16.0 \pm 1.29 \, \text{mg N kg}^{-1}$).

Residue-derived C in soil fractions. Compared with the initial samples before incubation, the proportions of residue-derived C in the whole soil or lighter soil fractions (F1 and F2) were significantly lower, whereas higher in the heavier fraction (F3) at the conclusion of the experiment. Mineral N addition to the HOST soils significantly reduced the proportion of residue-derived C in the F1 fraction of their TEST soils from 78.5% in the no N input control (AMF−N) to 68.9% and 41.5% in the AMF + N and AMFs + N treatments, respectively (Table 4). No statistically significant differences among treatments were found regarding the proportions of residue-derived C in the heavier F2 and F3 fractions, and whole soil samples (Table 4).

Discussion

Nitrogen uptake and transport by AM fungal hyphae have been well documented (Ames *et al.*, 1983; Johansen *et al.*, 1992; Mäder *et al.*, 2000; Bago *et al.*, 1996; Hodge *et al.*, 2001; Tanaka & Yano, 2005), which were also demonstrated in our study using a ^{15}N tracer. However, what remains unclear is whether and how plant N status and mineral N inputs to the soil impact mycorrhizal contributions to plant nutrient (particularly N)

Table 4 The mean proportion (%) of the residue C in whole soil and soil density fractions from the TEST compartments before and 15 weeks after emergence of wild oat seedlings in the HOST compartments (Experiment 2)

Treatment	Whole soil	Soil density fractions		
		F1*	F2	F3
Before incubation	33.4a[†] (1.35)[‡]	93.5a (0.92)	40.8a (1.18)	6.93a (1.58)
After incubation				
AMF−N	14.3b (1.19)	78.5b (0.72)	27.8b (4.10)	11.3b (1.35)
AMF + N	13.5b (0.62)	68.9c (0.72)	23.4b (1.68)	10.4b (0.80)
AMFs + N	12.0b (0.83)	41.5d (4.74)	24.7b (3.65)	11.6b (0.58)

*F1, soil fraction with density $(d) < 1.0 \, g \, cm^{-3}$; F2, soil fractions with d between 1.0 and $1.6 \, g \, cm^{-3}$; F3, soil fractions with $d > 1.6 \, g \, cm^{-3}$.

[†]Values followed by different letters in a column are significantly different at $P \leq 0.05$ (LSD).

[‡]Values in the parentheses are standard errors.

AMF−N, wild oat and single arbuscular mycorrhizal fungal species in the HOST compartment without N inputs; AMF + N, oat and single arbuscular mycorrhizal fungal species plus N in the HOST compartment; AMFs + N, oat and multiple arbuscular mycorrhizal fungal species plus N in the HOST compartment.

acquisition. Applications of mineral N often suppress root biomass (Staddon *et al.*, 2004) and reduce the percentage of root colonization by AM fungi (Ellis *et al.*, 1992; Ryan & Ash, 1999; Staddon *et al.*, 2004), likely reducing N transport by AM fungal hyphae (Johansen *et al.*, 1994; Mäder *et al.*, 2000). However, results from our present experiment showed that mineral N inputs to a N-poor soil stimulated growth of plant shoots and roots as well as total root length colonized by mycorrhizal fungi, resulting in enhanced hyphal N uptake and transport from the adjacent TEST soil to host plants. These results differ from previous studies likely due to differences in initial soil N availability. Plant N status can profoundly impact C allocation to roots, mycorrhizae, and mycorrhizal functions. Both organic C availability from the host plant and soil mineral nutrients can impact AM fungal growth (Treseder & Allen, 2002). When low soil N constrains plant growth, N inputs can enhance plant growth and the allocation of photosynthetic products belowground, benefiting AM fungal growth (Hawkins & George, 1999). However, when soil N is not a limiting factor for plant growth, plants may allocate less C belowground, restricting mycorrhizal growth. In other words, sufficient N supply to the plant is important for the development of an extensive mycelium (Hawkins & George, 1999; Treseder & Allen, 2002), but excessive N may reduce plant C allocation belowground and consequently reduce host root availability needed for mycelium development (Ericsson, 1995).

Results from our present study also indicate that stimulation of AM fungal growth by N inputs can enhance decomposition of newly added residues in soil (Table 4). Increased hyphal growth and functioning in the TEST soil could be inferred from measurements of significantly lower extractable N concentrations in the TEST compartments of microcosms receiving N in the HOST compartment compared with the no-N-added controls. Mycorrhizal proliferation, especially in areas where the most active decomposition occurs (St John *et al.*, 1983; Aristizábal *et al.*, 2004), may facilitate residue decomposition by stimulating microbial activity through enhanced labile C availability from the host plant (Zak *et al.*, 2000; Nakano *et al.*, 2001). Results from an experiment by Hodge *et al.* (2001) using root exclusion and a dual $^{15}N/^{13}C$ labeling technique provide direct evidence of mycorrhizal enhancement of litter decomposition by showing that ^{13}C content in the samples from the AM fungal hyphal compartment was significantly lower than in those from the control (no hyphal presence). In our study, stimulation of litter decomposition in the TEST soil with N additions to the HOST compartment, as indicated by a reduced amount of particulate organic C derived from added residues (Table 4), provides direct evidence demonstrating that N inputs can facilitate decomposition by enhancing mycorrhizal growth and possibly associated microbial activity.

Enhancement of residue decomposition by mycorrhizae has significant implications for understanding soil C dynamics and plant N acquisition in future conditions of simultaneously occurring increases in atmospheric N deposition and atmospheric CO_2 concentration. Elevated CO_2 often increases AM fungal growth (Treseder, 2004), which has been suggested to promote C storage in soil (Rillig, 2004). AM fungal growth directly enhances C inputs to the soil and aids in the stabilization of soil aggregates, which inhibits

mineralization of soil C (Miller & Jastrow, 1990; Tisdall *et al.*, 1997). Also, chitin, the major component of fungal cell walls (up to 60%) (Treseder & Allen, 2000), and glomalin, a glycoprotein produced by AM fungi, may contribute to C retention in soil because they can remain relatively undegraded in the soil for several years and even decades (Rillig *et al.*, 2001). The enhancement of residue decomposition by mycorrhizae observed in the current study and other experiments (Hodge *et al.*, 2001) differs from the conventional view regarding the extent to which mycorrhizae contribute to decomposition processes and C retention in soil. However, mycorrhizal enhancement of residue decomposition may still enhance ecosystem C sequestration because the resulting N release from decomposing organic residues may partially relieve N constraints on plant responses to rising atmospheric CO_2 levels in unmanaged and natural ecosystems (as opposed to managed agroecosystems where N levels are typically high) (Hu *et al.*, 2001; Oren *et al.*, 2001). These results also highlight the need for long-term field experiments to assess the net effect of mycorrhizae on C balance in various ecosystems.

It is interesting to note, multiple AM fungal species were more effective in mediating N transport and residue decomposition than the single AM fungal species. This suggests that results obtained using individual AM fungal species in many microcosm experiments may be difficult to extrapolate to field conditions where various mycorrhizal species coexist (Smith & Read, 1997; Egerton-Warburton & Allen, 2000). Different AM fungal species vary markedly in their root colonization and mycelium growth, leading to variation in their ability to supply plants with nutrients and promote plant growth (van der Heijden *et al.*, 1998; Smith *et al.*, 2000; van der Heijden *et al.*, 2003). For example, van der Heijden *et al.* (1998) showed that high diversity of AM fungal species enhanced AM fungal hyphal length and plant P content, but reduced soil P concentration. Also, Azcón *et al.* (2001) showed that *G. fasciculatum* was more efficient in NO_3^- uptake and translocation than *G. mosseae* because the former had a higher percentage root infection and more extensive hyphae. In addition, AM fungal species may exhibit spatial differences in acquisition of soil nutrients. Smith *et al.* (2000) observed that plants colonized by *Scutellospora calospora* preferentially acquired P from the soil closer to the roots than those colonized by *G. caledonium*. The coexistence of multiple AM fungal species likely leads to complementary and additive effects (van der Heijden *et al.*, 1998; van der Heijden *et al.*, 2003). Together, these results indicate that the composition of mycorrhizal species can significantly impact experimental results, and extreme care is advised when extrapolating results obtained from microcosm experiments with a single AM fungal species to field conditions.

In summary, results obtained from our microcosm experiments showed that low mineral N inputs to a N-poor soil enhanced mycorrhizally mediated N acquisition by host plants and stimulated residue decomposition in the soil, although increased AM fungal hyphae could contribute C inputs to soil. These findings suggest that mycorrhizae can have contrasting effects on ecosystem C balance depending on soil N availability. Long-term field experiments are needed to understand the role of mycorrhizae and their net impact on soil C dynamics, particularly under future conditions of concurrent increases in atmosphere CO_2 concentration and N deposition.

Acknowledgements

References

Ames RN, Reid CPP, Porter LK *et al.* (1983) Hyphal uptake and transport of nitrogen from two ^{15}N-labelled sources by *Glomus mosseae*, a vesicular-arbuscular mycorrhizal fungus. *New Phytologist*, **95**, 381–396.

Aristizábal C, Rivera EL, Janos DP (2004) Arbuscular mycorrhizal fungi colonize decomposing leaves of *Myrica parvifolia*, *M. pubescens* and *Paepalanthus* sp. *Mycorrhiza*, **14**, 221–228.

Azcón R, Ruiz-Lozano JM, Rodríguez R (2001) Differential contribution of arbuscular mycorrhizal fungi to plant nitrate uptake (^{15}N) under increasing N supply to the soil. *Canadian Journal of Botany*, **79**, 1175–1180.

Aziz T, Habte M (1989) Influence of inorganic-N on mycorrhizal activity, nodulation, and growth of *Leucaena leucocephala* in an oxisol subjected to simulated erosion. *Communications in Soil Science and Plant Analysis*, **20**, 239–251.

Bago B, Vierheilig H, Piché Y *et al.* (1996) Nitrate depletion and pH changes induced by the extraradical mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* grown in monoxenic culture. *New Phytologist*, **133**, 273–280.

Baisden WT, Amundson R (2002) Turnover and storage of C and N in five density fractions from California annual grassland surface soils. *Global Biogeochemical Cycles*, **16**, 64/61–64/16.

Buwalda JG, Goh KM (1982) Host-fungus competition for carbon as a cause of growth depressions in vesicular-arbuscular mycorrhizal ryegrass. *Soil Biology and Biochemistry*, **14**, 103–106.

Chapin FS III, Matson PA, Mooney HA (2002) *Principles of Terrestrial Ecosystem Ecology.* Springer-Verlag, New York, NY, USA.

Clark RB, Zeto SK (2000) Mineral acquisition by arbuscular mycorrhizal plants. *Jornal of Plant Nutrition*, **23**, 867–902.

Corkidi L, Rowland DL, Johnson NC *et al.* (2002) Nitrogen fertilization alters the functioning of arbuscular mycorrhizas at two semiarid grasslands, **240**, 299–310.

Dhillion SS, Ampornpan L (1992) The influence of inorganic nutrient fertilization on the growth, nutrient composition and vesicular-arbuscular mycorrhizal colonization of pretransplant rice (*Oryza sativa* L.) plasnts. *Biology and Fertility of Soils*, **13**, 85–91.

Egerton-Warburton LM, Allen EB (2000) Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. *Ecological Applications*, **10**, 484–496.

Ellis JR, Roder W, Mason SC (1992) Grain-sorghum soybean rotation and fertilization influence on vesicular-arbuscular mycorrhizal fungi. *Soil Science Society of America Journal*, **56**, 789–794.

Ericsson T (1995) Growth and shoot–root ratio of seedlings in relation to nutrient availability. *Plant and Soil*, **169**, 205–214.

Fixen PE, West FB (2002) Nitrogen fertilizers: meeting contemporary challenges. *AMBIO*, **31**, 169–176.

Giovannetti M, Mosse B (1980) Evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist*, **84**, 489–500.

Gough L, Osenberg CW, Gross KL *et al.* (2000) Fertilization effects on species density and primary productivity in herbaceous plant communities. *Oikos*, **89**, 428–439.

Hart SC, Stark JM, Davidson EA *et al.* (1994) Nitrogen mineralization, immobilization, and nitrification. In: *Methods of Soil Analysis. Part 2: Microbiological and Biochemical Properties* (eds Weaver RW *et al.*), pp. 985–1018. Soil Science Society of America Inc., Madison, WI, USA.

Hawkins HJ, George E (1999) Effect of plant nitrogen status on the contribution of arbuscular mycorrhizal hyphae to plant nitrogen uptake. *Physiologia Plantarum*, **105**, 694–700.

Hodge A, Campbell CD, Fitter AH (2001) An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature (London)*, **413**, 297–299.

Hu S, Chapin FS III, Firestone MK *et al.* (2001) Nitrogen limitation of microbial decomposition in a grassland under elevated CO_2. *Nature (London)*, **409**, 188–191.

Jakobsen I, Rosendahl L (1990) Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. *New Phytologist*, **115**, 77–83.

Johansen A, Jakobsen I, Jensen ES (1992) Hyphal transport of ^{15}N-labelled nitrogen by a vesicular-arbuscular mycorrhizal fungus and its effect on depletion of inorganic soil N. *New Phytologist*, **122**, 281–288.

Johansen A, Jakobsen I, Jensen ES (1994) Hyphal N transport by a vesicular-arbuscular mycorrhizal fungus associated with cucumber grown at three nitrogen levels. *Plant and Soil*, **160**, 1–9.

Littell RC, Milliken GA, Stroup WW *et al.* (1996) *SAS System for Mixed Models.* SAS Institute, Inc, Cary, NC.

Mäder P, Vierheilig H, Streitwolf-Engel R *et al.* (2000) Transport of ^{15}N from a soil compartment separated by a polytetrafluoroethylene membrane to plant roots via the hyphae of arbuscular mycorrhizal fungi. *New Phytologist*, **146**, 155–161.

Miller RM, Jastrow JD (1990) Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. *Soil Biology and Biochemistry*, **22**, 579–584.

Nakano A, Takahashi K, Kimura M (2001) Effect of host shoot clipping on carbon and nitrogen sources for arbuscular mycorrhizal fungi. *Mycorrhiza*, **10**, 287–293.

Oren R, Ellsworth DS, Johnsen KH *et al.* (2001) Soil fertility limts carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. *Nature (London)*, **411**, 469–472.

Phillips DL, Gregg JW (2001) Uncertainty in source partitioning using stable isotopes. *Oecologia*, **127**, 171–179.

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, **55**, 158–161.

Read DJ, Perez-Moreno J (2003) Mycorrhizas and nutrient cycling in ecosystems–a journey towards relevance? *New Phytologist*, **157**, 475–492.

Rillig MC (2004) Arbuscular mycorrhizae and terrestrial ecosystem processes. *Ecology Letters*, **7**, 740–754.

Rillig MC, Wright SF, Nichols KA *et al.* (2001) Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. *Plant and Soil*, **233**, 167–177.

Ryan M, Ash J (1999) Effects of phosphorus and nitrogen on growth of pasture plants and VAM fungi in SE Australian soils with contrasting fertiliser histories (conventional and biodynamic). *Agriculture, Ecosystems & Environment*, **73**, 51–62.

Smith FA, Jakobsen I, Smith SE (2000) Spatial differences in acquisition of soil phosphate between two arbuscular mycorrhizal fungi in symbiosis with *Medicago truncatula*. *New Phytologist*, **147**, 357–366.

Smith SE, Read DJ (1997) *Mycorrhizal Symbiosis.* Academic Press, San Diego, CA, USA.

St John TV, Coleman DC, Reid CPP (1983) Association of vesicular-arbuscular mycorrhizal hyphae with soil organic particles. *Ecology*, **64**, 957–959.

Staddon PL, Jakobsen I, Blum H (2004) Nitrogen input mediates the effect of free-air CO_2 enrichment on mycorrhizal fungal abundance. *Global Change Biology*, **10**, 1678–1688.

Tanaka Y, Yano K (2005) Nitrogen delivery to maize via mycorrhizal hyphae depends on the form of N supplied. *Plant, Cell and Environment*, **28**, 1247–1254.

Tinker PB, Nye PH (2000) *Solute Movement in the Rhizosphere.* Oxford University Press, New York, NY, USA.

Tisdall JM, Smith SE, Rengasamy P (1997) Aggregation of soil by fungal hyphae. *Australian Journal of Soil Research*, **35**, 55–60.

Treseder KK (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. *New Phytologist*, **164**, 347–355.

Treseder KK, Allen MF (2000) Mycorrhizal fungi have a potential role in soil carbon storage under elevated CO_2 and nitrogen deposition. *New Phytologist*, **147**, 189–200.

Treseder KK, Allen MF (2002) Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. *New Phytologist*, **155**, 507–515.

USDA-NASS, (2004) Agricultural Chemical Usage: 2003 field crops summary. May 2004. Ag Ch 1 (04) a, 163 pp.

van der Heijden MGA, Klironomos JN, Ursic M *et al*. (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature (London)*, **396**, 69–72.

van der Heijden MGA, Wiemken A, Sanders IR (2003) Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occurring plant. *New Phytologist*, **157**, 569–578.

Wedin DA, Tilman D (1996) Influence of nitrogen loading and species composition on the carbon balance of grasslands. *Science*, **274**, 1720–1723.

Wright SF, Upadhyaya A (1998) A survey of soils for aggregate stability and glomatin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil*, **198**, 97–107.

Zak DR, Pregitzer KS, King JS (2000) Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. *New Phytologist*, **147**, 201–222.